

**WHAT IS CLAIMED IS:**

1. A method for preparing a polymer conjugate, said method comprising:
  - (a) providing a water-soluble polymer comprising a maleimide group,
  - (b) reacting said polymer with an active agent comprising a nucleophile under conditions effective to couple said agent to said water soluble polymer via a Michael-type addition reaction to form a polymer-succinimide-linked active agent conjugate, and
  - (c) treating the conjugate from (b) under conditions effective to force open said succinimide ring to thereby form a polymer-conjugate composition comprising a polymer-succinamic acid-conjugate.
- 10 2. The method of claim 1, wherein said treating step comprises hydrolysis.
3. The method of claim 2, wherein said treating is carried out in an aqueous or an organic solvent.
- 15 4. The method of claim 1, wherein said treating step is carried out in the presence of base.
5. The method of claim 4, wherein said base is selected from the group consisting of metal or non-metal hydroxides, quaternary ammonium hydroxides, sodium ( $\text{Na}^{\circ}$ ), and potassium ( $\text{K}^{\circ}$ ).
6. The method of claim 4, wherein said base is on a solid support or in solution.
- 25 7. The method of claim 1, wherein said treating step is carried out at pHs ranging from about 6 to 12.
8. The method of claim 7, wherein said treating step is carried out at pHs ranging from about 7.5 to about 11.

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9. The method of claim 1, wherein said treating step is carried out in a buffer.
10. The method of claim 1, wherein said treating is carried out under conditions effective to provide a chemically stable composition.
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11. The method of claim 1, further comprising the step of determining the extent of opening of said succinimide ring in said composition.
12. The method of claim 11, wherein said treating is carried out until at least about  
10 15% of the polymer-succinamic acid-conjugate is formed.
13. The method of claim 11, wherein said treating is carried out until at least about  
35% of said polymer-succinamic acid-conjugate is formed.
- 15 14. The method of claim 11, wherein said treating is carried out until at least about  
80% of said polymer-succinamic acid-conjugate is formed.
15. The method of claim 11, wherein said treating is carried out until at least about  
95% of said polymer-succinamic acid-conjugate is formed.
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16. The method of claim 11, wherein said treating is carried out until at least about  
98% of said polymer-succinamic acid-conjugate is formed
17. The method of claim 1, wherein said nucleophile is a sulfhydryl (thiol) group or  
25 an amino group.
18. The method of claim 1, wherein said active agent is a protein or a peptide.

19. The method of claim 1, further comprising recovering said polymer succinamic acid conjugate from the composition.
20. The method of claim 19, wherein said recovering step comprises precipitating  
5 said polymer succinamic acid conjugate.
21. The method of claim 19, wherein said recovering step further comprises purifying said polymer succinamic acid conjugate.
- 10 22. The method of claim 21, wherein said purifying step comprises purifying said polymer succinamic acid conjugate by chromatography.
- 15 23. The method of claim 22, wherein said chromatography is selected from the group consisting of SDS-PAGE, gel permeation chromatography, and ion exchange chromatography.
24. The method of claim 1, wherein said water soluble polymer is selected from the group consisting of a poly(alkylene oxide), poly(vinyl pyrrolidone), poly(vinyl alcohol), polyoxazoline, poly(acryloylmorpholine), and poly(oxyethylated polyol).
- 20 25. The method of claim 24, wherein said water soluble polymer is a poly(alkylene oxide).
- 25 26. The method of claim 25, wherein said water soluble polymer is a poly(ethylene glycol).
27. The method of claim 26, wherein the poly(ethylene glycol) comprises an end-capping moiety.

28. The method of claim 27, wherein the end-capping moiety is selected from the group consisting alkoxy, substituted alkoxy, alkenyloxy, substituted alkenyloxy, alkynyloxy, substituted alkynyloxy, aryloxy, and substituted aryloxy.

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29. The method of claim 28, wherein the end-capping moiety is selected from the group consisting of methoxy, ethoxy, and benzyloxy.

30. The method of claim 26, wherein the poly(ethylene glycol) has a nominal average  
10 molecular mass of from about 100 daltons to about 100,000 daltons.

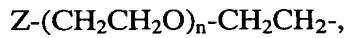
31. The method of claim 30, wherein the poly(ethylene glycol) has a nominal average  
molecular mass of from about 1,000 daltons to about 80,000 daltons.

15 32. The method of claim 31, wherein the poly(ethylene glycol) has a nominal average  
molecular mass of from about 2,000 daltons to about 50,000 daltons.

33. The method of claim 26, wherein said poly(ethylene glycol) has a structure  
selected from the group consisting of linear, branched, forked, and multi-armed.

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34. The polymer of claim 26, wherein said poly(ethylene glycol) comprises the  
structure:



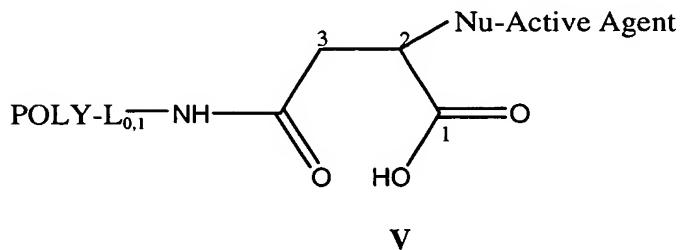
25 where n is from about 10 to about 4000, and Z comprises a moiety selected from  
the group consisting of hydroxy, amino, ester, carbonate, aldehyde, aldehyde hydrate,  
acetal, ketone, ketone hydrate, ketal, alkenyl, acrylate, methacrylate, acrylamide, sulfone,  
thiol, carboxylic acid, isocyanate, isothiocyanate, hydrazide, urea, maleimide,  
vinylsulfone, dithiopyridine, vinylpyridine, iodoacetamide, alkoxy, benzyloxy, silane,  
30 lipid, phospholipid, biotin, and fluorescein.

35. The method of claim 1, wherein said water-soluble polymer comprises a linker, L, interposed between said water-soluble polymer and said maleimide group.
36. The method of claim 35, wherein said linker is effective to result in a ring-opening hydrolysis half-life of said water-soluble polymer of about 12 hours or less when measured at room temperature in phosphate buffer at pH 9.0.
37. The method of claim 26, wherein said polyethylene glycol polymer is directly attached to the nitrogen atom of said maleimide group.
- 10 38. A polymer conjugate composition prepared according to the method of claim 1.
39. A composition comprising a water-soluble polymer covalently attached to a maleamic acid group, optionally via an interposing linker.
- 15 40. A composition comprising at least about 50% by weight of a water-soluble polymer covalently attached to a maleamic acid group, optionally via an interposing linker.
- 20 41. A composition of claim 39 that is chemically stable.
42. A composition of claim 39 that is resistant to hydrolysis.
43. The composition of claim 39, wherein said water soluble polymer is selected from the group consisting of a poly(alkylene oxide), poly(vinyl pyrrolidone), poly(vinyl alcohol), polyoxazoline, poly(acryloylmorpholine), and a poly(oxyethylated polyol).

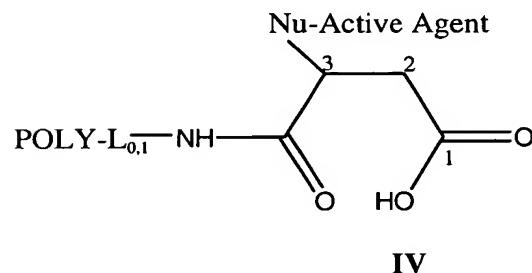
44. The composition of claim 39, wherein said maleamic acid group is covalently linked to a nucleophile of an active agent to form the corresponding succinamic acid derivative.

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45. A chemically stable composition comprising:



10                  or



wherein:

15                  POLY is a water-soluble polymer segment,

L is an optional linker, and

"Nu-Active agent" represents an active agent comprising a nucleophile,  
"Nu".

20    46. The composition of claim 45, wherein said Nu is thiol or thiolate.

47. The composition of claim 46, wherein said Nu is a thiol or thiolate contained in a cysteine and said active agent is a protein or peptide.

5 48. The composition of claim 45, wherein Nu is amino.

49. The composition of claim 45, wherein Nu is an amino group contained in a lysine or is a terminal amine and said active agent is a protein or peptide.

10 50. The composition of claim 45 in powder form.

51. The composition of claim 45 in solution form.

52. The composition of claim 45, comprising at least about 15% by weight of  
15 combined structures V and IV, based upon POLY-containing components.

53. The composition of claim 45, comprising at least about 35% by weight of  
combined structures V and IV, based upon POLY-containing components.

20 54. The composition of claim 45, comprising at least about 80% by weight of  
combined structures V and IV, based upon POLY-containing components.

55. The composition of claim 45, comprising at least about 95% by weight of  
combined structures V and IV, based upon POLY-containing components.

56. The composition of claim 45, comprising at least about 98% by weight of combined structures V and IV, based upon POLY-containing components.
57. The composition of claim 45, wherein L is a linker effective to provide an increased ring opening hydrolysis rate of the maleimide group of the uncoupled water soluble polymer maleimide precursor to either V or IV relative to that of the same water soluble polymer maleimide precursor but absent a linker.
58. The composition of claim 45, wherein L is a linker effective to result in a ring-opening hydrolysis half-life of the uncoupled water-soluble polymer maleimide precursor to either V or IV of about 12 hours or less when measured at room temperature in phosphate buffer at pH 9.
59. The composition of claim 45, wherein POLY comprises a polyethylene glycol and structures V and IV are absent a linker.
60. The composition of claim 59, wherein POLY is a linear polyethylene glycol.
61. The composition of claim 45, wherein said linker comprises an electron withdrawing group (EWG) within about 6 atoms of the nitrogen atom of said succinamic acid.
62. The composition of claim 61, wherein said linker comprises an electron withdrawing group (EWG) within about 3 atoms of the nitrogen atom of said succinamic acid.

63. The composition of claim 45, wherein said active agent is a biologically active agent.

64. A unit dosage form comprising the composition of claim 63.

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65. A protein derivatized with a water-soluble polymer, wherein the polymer is coupled to the protein via succinimide groups covalently attached to either cysteine sulphydryl groups or lysine amino groups, and substantially all of the succinimide groups are present in a ring-opened form.

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